A new method for measuring antioxidant activity

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A spectrophotmetric method for the measurement of antioxidant activity has been developed, based on the inhibition by antioxidants of the $ABTS^{\bullet+}$ radical cation, which has a characteristic long wave absorption spectrum showing maxima at 660 nm, 734 nm, and 820 nm. $ABTS^{\bullet+}$ can be generated by the interaction of ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonate) with the ferrylmyoglobin radical species, using ABTS (150 μM), H_2O_2 (75 μM) and metmyoglobin (2.5 μM) [1]. Antioxidant compounds suppress the $ABTS^{\bullet+}$ to an extent and on a time scale dependent on the antioxidant capacity of the substance under investigation. With automated timing and reagent additions, the precise and stable assay has application to the measurement of the antioxidant activity of pure solutions and of mixtures of substances in a wide range of pharmacological and physiological situations [2]. 3 μl of sample is required, which is mixed with ABTS-metmyoglobin reagent, and the reaction started with H_2O_2. The absorbances are read 6 minutes post mixing at 734 nm, and a calibration curve is constructed using Trolox solutions of known concentration.

Solutions of antioxidant substances can be compared to Trolox solutions by means of this assay, and hence to one another on the basis of their molar antioxidant activity. A mean figure is derived for the antioxidant capacity per mole of substance or "TEAC" (Trolox Equivalent Antioxidant Capacity) based on results derived from several separate assays. The TEAC value is defined as the millimolar concentration of a Trolox solution having the same antioxidant capacity as a 1.0 mM solution of the substance. Derivation of a TEAC value gives a direct comparative measure of the antioxidant capacity among groups of substances, for example drugs that are being evaluated as potential antioxidants.

Table 1 shows that the plasma antioxidants urate, ascorbate and α-tocopherol have the same antioxidant capacity as Trolox, as expected. Bilirubin has an antioxidant capacity 50% greater (on a mole for mole basis) than these substances. Albumin has a lower antioxidant capacity. The thiol compounds from glutathione to cysteine in the table exhibit widely varying activities, with N-acetyl cysteine as the most effective antioxidant. A number of experimental hydroxamate drugs were also compared. Desferrioxamine, with three potential hydrogen-donating centres [3], has a TEAC value of $\approx$3, suggesting that each hydroxamate group is capable of acting as a hydrogen donor. The monohydroxamates [4] give a proportionate value of $\approx$1. Urea and heparin (measured at 10,000 i.u.) do not function as antioxidants under these conditions and heparin can therefore be used for the collection of plasma without influencing the total antioxidant value of the sample.

TABLE 1

TEAC results for solutions of pure antioxidants: the TEAC = the mM concentration of a Trolox solution having the antioxidant capacity equivalent to a 1.0 mM solution of the substance under investigation.

Substance	TEAC	n	S.D.
bilirubin	1.50	3	0.12
urate	1.02	5	0.06
ascorbate	0.99	5	0.04
α-tocopherol	0.97	3	0.01
albumin	0.63	3	0.02
glutathione	0.90	3	0.03
N-acetylcysteine	1.43	3	0.08
N-mercapto-propionylglycine	0.57	3	0.03
penicillamine	0.38	3	0.06
cysteine	0.28	3	0.09
desferrioxamine (trihydroxamate)	2.96	4	0.09
N-methyl-mono-hydroxamates	1.21	2	

Glucose and mannitol do not act as antioxidants, although they have been reported [5] to react with the hydroxyl radical under specific conditions, and neither does ethanol, which can therefore be used to solubilise substances for inclusion in the assay. This method for measuring a TEAC value is dependent on the substance being water soluble, or on it being solubilised or emulsified with a detergent. The activity of alpha tocopherol can be determined by dissolving it in ethanol, and then emulsifying with 2 % aqueous Nonidet P-40 (a non-ionic detergent) before introducing it into the reaction system.

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